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(71) Applicants *(for all designated States except US)*: UNIVERSITY OF TORONTO [CA/CA]; Medical Sciences Building, 1 Kings College Circle, Toronto, Ontario M5S 1A8 (CA). CONNAUGHT LABORATORIES LIMITED [CA/CA]; 1755 Steeles Avenue West, Toronto, Ontario M2R 3T4 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: PAI, Emil, F. [DE/CA]; 51 Dunedin Drive, Toronto, Ontario M8X 2K7 (CA). KLEIN, Michel, H. [CA/CA]; 16 Munro Boulevard, Willowdale, Ontario M2P 1B9 (CA). CHONG, Pele [CA/CA]; 32 Estoril Street, Richmond Hill, Ontario L4C 0B6 (CA). PEDYCZAK, Arthur [CA/CA]; 1399 Colmar Avenue, Pickering, Ontario L1W 1C2 (CA).

(74) Agent: STEWART, Michael, L.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

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(54) Title: Fab–EPITOPE COMPLEX FROM THE HIV–1 CROSS–NEUTRALIZING MONOCLONAL ANTIBODY 2F5

(57) Abstract

The crystal structure of the Fab' fragment of Mab 2F5, a potent neutralizer of both laboratory strains and primary clinical isolates of most clades of HIV–1, both uncomplexed and complexed with the largely conserved peptide sequence ELDKWAS of the viral envelope protein gp41, has been elucidated and the characteristics of peptide–protein interactions determined. Having regard to such determination, the peptide–mimetics are constrained in the three–dimensional structure to provide an increased immunogenicity to the epitope sequence.

The elucidation of these three-dimensional structures enables there to be constructed, as set forth herein, peptide-mimetics constrained in the same β-turn-like configuration as seen in the crystal structure of the complex, which would be expected to increase the immunogenicity of the epitope sequence.

Accordingly, in one aspect of the invention, there is provided an isolated crystal of the Fab' fragment of monoclonal antibody 2F5. The isolation of the crystalline form of the Fab'2F5 fragment enables the three-dimensional structure of such form of the fragment to be determined and such structure is shown in Figure 1, described below. Certain characterizing parameters have been determined for the crystal structure, as set forth in Table 2 below.

The isolated crystal may be grown in space group $P2_12_12_1$ with cell dimensions a=63.6 Å; b=76.4 Å; c=93.4 Å, although the crystals may be grown in another space group with its own unique cell dimensions. The crystalline form of the Fab'2F5 may have the atomic coordinates deposited on April 9, 1999 with the Protein Data Bank under Accession No. 2F5A.

Fab'2F5 molecules organized in the isolated crystal provided herein possess a third hypervariable (V3) loop of the heavy chain comprising amino acid residues H98 to H120, as seen in Table 1 below, which has a three-dimensional structure as shown in Figure 4, described below and atomic coordinates as shown in Table 3 below.

In accordance with a further aspect of the present invention, there is provided an isolated crystal of the Fab' fragment of monoclonal antibody 2F5 complexed with a peptide having the amino acid sequence ELDKWAS (SEQ ID No: 1) or a functional analog thereof. The solution of the crystal form of the complex enables the three-dimensional structure of such form of the complex to be determined and the detail of the binding site of the peptide to the Fab' fragment is shown in Figure 3, described below. Certain characterizing parameters have been determined for the crystal structure of the complex, as set forth in Table 2 below.

The isolated crystal complex may be grown in space group $P2_12_12_1$ with cell dimensions a=58.0 Å; b=65.0 Å; c=175.6 Å, although the crystal complex may be grown in another space group with its own unique cell dimensions. The

crystalline form of the complexed form of the Fab'2F5 may have the atomic coordinates deposited with the Protein Data Bank under Accession No. 2F5B on April 9, 1999.

The functional analog of the amino acid sequence ELDKWAS may be one in which lysine is replaced by arginine and/or one in which tryptophan is replaced by tyrosine, phenylalanine or uncharged histadine. One example of such functional analog is ELDRWAS (SEQ ID No: 2).

The elucidation of the crystal structure of the Fab'2F5 fragment when bound to the peptide ELDKWAS (SEQ ID No: 1), provides details of the actual conformation of the peptide epitope when it is bound to the antibody, which will be the same, irrespective of the kind of crystal which is analyzed.

The information which is provided concerning the conformation of peptide epitope then provides the basis for the provision of peptide analogs, peptide mimetics and other antigens which are potentially useful as components of an anti-HIV vaccine.

Accordingly, in another aspect of the present invention, there is provided a synthetic peptide which binds to monoclonal antibody 2F5 and which is constrained to provide a three-dimensional structure corresponding to that for the peptide ELDKWAS (SEQ ID No: 1) shown in Figure 3.

This synthetic peptide may contain the amino acid sequence DKW or a functional analog thereof and may be constrained in the slightly distorted β-turn configuration of the three-dimensional structures with the tryptophan and lysine residue chains stacked and parallel, as seen in Figure 3 and as discussed in more detail below.

The analysis of the three-dimensioned conformation of the epitope indicates that at least one of the tryptophan and lysine sidechains may be substituted by an amino acid which retains the peptide-protein interaction shown in Figure 3, which also binds to the Mab. For example, arginine (R) may be used in place of lysine (K) and tyrosine (Y), phenylalanine (F) and uncharged histadine (H) may be used in place of tryptophan (W). Peptides wherein one or more of such amino acid substitution is effected are peptides which contain a "functional

analog" of the amino acid sequence DKW, as the term is understood herein, in that the peptide still binds to the monoclonal antibody 2F5.

The synthetic peptide provided herein may be constrained in the required conformation by any convenient means. For example, a disulphide bridge may be used to maintain the amino acid sequence DKW or analogs thereof in the respective orientation of two amino acid residues as shown in Figure 3. Such disulphide bridge may be provided between cysteine residues in the synthetic peptide ECDKWCS (SEQ ID No.: 3).

Alternatively, a lactam bond may be used to maintain the amino acid sequence DKW or functional analogs thereof in the respective orientation of the amino acid residues as shown in Figure 3. Such lactam bond may be formed between diaminopropionic acid (Dap) and glutamate (E) residues in the synthetic peptide EdapDKWES (SEQ ID No.: 4) or EEDKWDapS (SEQ ID No.: 5).

It is well known that the immunogenicity of peptides may be enhanced by conjugation to carrier molecules, such as proteins, including diphtheria toxoid, tetanus toxoid or an outer membrane protein of *Haemophilus*. Such carrier protein may be linked to the peptide.

There is also provided, in an additional aspect of the invention, a method of making a peptide binding to monoclonal antibody 2F5, which comprises co-crystallizing a Fab' fragment of the monoclonal antibody 2F5 with a peptide having the amino acid sequence ELDKWAS (SEQ ID No.: 1) or functional analog thereof to form a crystalline complex; analyzing the crystalline complex to determine the three-dimensional orientation of the bound peptide in relation to the Fab' fragment; and synthesizing a peptide containing at least amino acids DKW or functional analogs thereof constrained in the determined three-dimensioned orientation.

The functional analog of the peptide containing at least amino acids DKW is one which still binds to the monoclonal antibody 2F5. Functional analogs also extend to known analogs of the ELDKWAS motif, including those of the formula X_1LDKWX_2S wherein X_1 is E, A, G or Q and X_2 is A or T.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent application contains drawings executed in color, namely Figures 1 to 4. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 is a colored ribbon diagram of crystalline Fab'2F5, showing the heavy chain in purple, the light chain in blue and the elongated VH3 loop (colored in gold) extending from the protein surface, as generated by MOLSCRIPT (ref. 27) and Raster 3D (ref. 28);

Figure 2 is a colored stereoplot of the ELDKWAS peptide model in density, as generated by the program O (ref. 29). The Fo–Fc map was calculated with the peptide omitted and contoured at 3σ. A minor break in the density at P7-Ser at the contour level illustrates the slight increase in flexibility at the extremes of the bound epitope;

Figure 3 is a color representation of the antigen binding site of Fab'2F5, showing protein/peptide interactions, as generated using the program SETOR (ref. 30). The residues are colored by atom type: oxygen is red, nitrogen is blue, carbon is grey and sulfur is yellow. For clarity, some hydrophobic sidechains which interact with the epitope have been omitted. All bond lengths are given in Å; and

Figure 4 is a color representation of the third hypervariable loop of the heavy chain of Fab'2F5 complex comprising amino acid residues H98 to H120, as generated using the program SETOR (ref. 30). The residues are colored by atom type.

GENERAL DESCRIPTION OF INVENTION

The crystalline structure of the Fab' fragment of Mab 2F5 (IgG) was solved at 2.05 Å resolution by molecular replacement and adopts the standard immunoglobulin fold. A salient feature of the structure is the elongated (22 amino acids) hypervariable loop 3 of the heavy chain (V-H3, ref. 9), which comprises amino acid residues H98 to 120 and extends away from the protein surface, as can be seen from the ribbon diagram of Figure 1. The V-H3 loop is shown in detail in Figure 4. The atomic coordinates of the V-H3 loop are given in Table 3.

In the structure of the Fab'2F5 complex with bound epitope, refined at 2.0 Å, this loop is well-defined by clear electron density. In the uncomplexed form, while the V-H3 region is less clear, loops at the C-terminal regions of the heavy chain constant domain, including the C-termini of both chains, were better resolved. Conformations from the better-defined electron density were used as templates for building the other model. The refined models comprise residues L1 to L214 of the light chain and residues H1 to H146 and H151 to H235 of the heavy chain plus ordered water molecules. The amino acid sequences of the light chain (SEQ ID No.: 2) and heavy chain (SEQ ID No.: 3) of Fab'2F5 are shown in Table 1 below. For the structure of the complex, P1 to P7 are the residues of the peptide. The H147 to H150 loop of the constant domain of the heavy chain was not visible in either structure. (Residues are labelled herein H1 to H235 for the heavy chain and L1 to L214 for the light chain and P1 to P7 for the peptides).

Along with differences in mobility of the loops mentioned above, the elbow angle in the complexed form differs from uncomplexed Fab'2F5 (142° vs. 146°). Both of these observations may be artifacts of crystal packing, since the unit cells are different, uncomplexed Fab'2F5 having a unit cell which is 30% smaller. An overlay of all Cα atoms results in an rmsd of 0.7 Å, but these shifts appear to be the result of a concerted domain movement (i.e. the change in elbow angle) rather than any modification of the antigen binding site. Superpositioning only the variable regions gives an rmsd of 0.4 Å. While the results of the structural analysis do not provide any obvious explanation for the long insertion in the V-H3 loop has been identified, its unusually hydrophobic nature for surface residues suggests it plays a role in the antibody mechanism. It may be involved in interactions with a portion of gp41 C-terminal to the epitope sequence, enhancing binding and increasing the specificity of the Fab. It may even form an integral part of the neutralization mechanism, perhaps by disrupting the conformation of the gp41 coiled-coil trimer.

In the complexed structure, the ELDKWAS peptide forms a slightly distorted, type I β turn, centered between P4-Lys and P5-Trp, (as seen in Figures 2 and 3), with a 3.1 Å hydrogen bond from the amide nitrogen of P6-Ala to the carbonyl oxygen of P3-Asp. The arrangement is atypical in that neither position

two or three in the turn is a glycine (ref. 10), but rather the bulky residues lysine and tryptophan. The dihedral angles for P5-Trp fall in the "unfavoured" region of a Ramachandran plot (ϕ=-101.7°, ψ=8.7°).

Another interesting feature of the complexed structure is the stacked arrangement of the adjacent P5-Trp and P4-Lys sidechains, with hydrophobic interactions between the fully-extended alkyl chain of the P4-Lys and the aromatic rings of P5-Trp at a distance of about 3.8 Å. The lysine sidechain, whose hydrophobic methylene groups are sandwiched between P5-Trp and H54-Tyr, ends with a sharp turn at the final amino group, forming contacts with H56-Asp and H58-Asp. While the principal hydrophobic contacts of P5-Trp are the P4-Lys methylene groups, other hydrophobic residues within 4 Å of the aromatic ring system include H103-Pro and H32-Phe and the methylene groups of the sidechain of H113-Arg. A key component to the stability of the peptide configuration is the orientation of the P3-Asp sidechain, which forms strong hydrogen bonds to the backbone amide of P5-Trp as well as to L96-His-Nε and H100-Arg-NH1, all about 2.8 Å long. A water molecule associated with P5-Trp-Nε1 at 3.0 Å also forms strong hydrogen bonds to backbone carbonyls of H33-Gly and H101-Arg at 2.7 and 2.8 Å respectively. From this analysis, it can be concluded that the Asp-Lys-Trp (DKW) trio are the essential component of the protein/peptide interaction.

This conclusion is supported by mutation studies in which changes outside the DKW core do not have a dramatic effect on binding, whereas major modifications within the trio usually prevent neutralization (ref. 5). It was estimated that the LDKW motif is 83% conserved among HIV-1 envelope glycoprotein sequence (ref. 4). For the critical portion of the epitope, DKW, conservation among 206 sequenced HIV-1 envelope proteins of all clades in the 1997 to 1998 Los Alamos HIV Sequence Database (ref. 11) is 86%. Within the B clade, conservation is 92% (91/99 sequences). Phage library screening with Mab 2F5 also selected sequences with a DRW core (ref. 4). The structure of a complex where an arginine is substituted for P4-Lys (i.e. peptide ELDRWAS (SEQ ID No: 2)) shows identical peptide conformation and contacts as the complex reported here with the consensus epitope. The total buried accessible surface area upon

formation of the complex is 1025 Å^2 (calculated as the difference in accessible surface between the intact complex and the sum of the surface areas of the peptide and uncomplexed Fab' determined using a probe of radius 1.4 Å (ref. 12)). The peptide coordinates of the complex Fab'2f5 + ELDKWAS are shown in Table 4 while those for the complex Fab'2f5 + ELDRWAS are shown in Table 5.

The conformation of the gp41 epitope found in the complex with Fab'2F5 and seen in detail in Figure 3 was not anticipated. A helical conformation had been proposed (ref. 13) which was consistent with an extension of the observed coiled coil of the gp41 ectodomain (refs. 14 to 19). Most structural analyses of HIV-1 (refs. 14 to 16) or SIV (refs. 17 to 19) gp41 do not incorporate the epitope sequence, although two reports (refs. 14, 19) include a partial sequence. In one (ref. 14), ELD at the C-terminus of the crystallized portion adopted an α-helical structure, the continuation of a long (37 aa) helix. In the other, the C-terminus is an unstructured coil (ref. 19).

A conformation of the full epitope was determined as part of a fusion protein, where it was connected to the C-terminus of glutathione-S-transferase (GST) by a nine amino acid linker (ref. 20). In this environment, the epitope formed part of a series of tight turns but not the β-turn seen in the results described herein. In the GST-fusion structure, the epitope peptide interacted with a neighboring molecule in the crystal, making it probable that crystal packing forces led to the observed conformation. The gp41 peptide portion of the structure also had high thermal parameters, denoting flexibility.

Preliminary NMR studies have suggested that the ELDKWAS sequence adopts very little or no stable secondary structure. The crystal structure of Fab'2F5 elucidated herein explains the stronger immune response observed when the epitope was introduced into loops of hemagglutinin (refs. 2, 21) or recombinant antibodies (ref. 22) where a β-turn conformation might be induced, in contrast to hepatitis B virus surface antigen (ref. 8), where epitope grafting resulted in an excellent humoral response of 2F5-like binding specificity but failed to neutralize live virus, underlining the importance of the correct epitope conformation.

The conformation of the gp41 epitope set forth herein may be adopted transiently, after assembly of the mature gp41/gp120 trimers on the virus

envelope, or possibly during the fusion process. A range of conformations for gp41, including the stable fusogenic form observed in the structural determinations made herein, as well as an intermediate "unsprung" and non-fusogenic form has been proposed by several investigators (refs. 14, 23). A short life span of the antigen would be consistent with its low immunogenicity and the consequent absence of Mab 2F5 in the antisera of most infected patients. As well, passive immunization with Mab 2F5 in chimpanzees failed to neutralize HIV-1, resulting in delayed infection and lower viral loads, but not protection (ref. 6). This result was presumably due to insufficient opportunity for antibody binding, either because of low antibody concentration or the short lifetime of the antigenic conformation. As the only identified cross-neutralizing antibody against gp41, Mab 2F5 is an important focus in HIV-1 vaccine research. It is one of only three broadly neutralizing monoclonal antibodies identified to date and the only one with a short, continuous epitope. The other two known cross-neutralizing Mab's are b12 and 2G12 which define epitopes at the CD4 binding site and V3/V4 loops of gp120 respectively (ref. 6), but in these cases the epitopes are discontinuous and involve both peptide and carbohydrate interactions (refs. 5, 6).

Development of a peptide-mimetic constrained to adopt the conformation of the gp41 sequence found in the structure of Fab'2F5 could overcome the low immunogenicity of the epitope, making such a compound a useful component of a future HIV-1 vaccine.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, peptide-mimetics chemistry, protein biochemistry, crystallography and immunology used but not explicitly described in

this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example shows the preparation, purification and crystallization of Fab'2F5 and its epitope complex.

Intact human IAM 2F5 IgG antibody was transformed into $F(ab')_2$ using standard pepsin protocols. $F(ab')_2$ was then stored with 1% (w/v) clinical human albumin added to the solution for stability. To separate the protein from the albumin, DE52 cellulose was swollen in 20mM Tris pH 8.0 and packed into a column 3 cm wide, 5 cm high, providing about 30 mL bed volume. The column was washed overnight with 2 L of 20 mM Tris pH 8.0.

55 ml protein at 1.1 mg/ml concentration were dialysed against 2 x 4 to 5 L of 20 mM Tris pH 8.0 and the conductivity and pH of the buffer, flow through and protein concentration were checked to ensure the protein bound to the column. The protein was loaded onto the column by pumping on at 1 to 5 mL/min, with albumen binding to the column while the F(ab')2 does not. Buffer A (20 mM Tris pH 8.0) was run through the column until the OD_{280} went down to baseline and approximately 7 mL fractions were collected.

The albumin was eluted with a salt gradient of 20 mM Tris pH 8.0, 20 mM Tris pH 8.0 + 0.2 M NaCl, to ensure no other proteins were present. The flow-through protein was concentrated, producing 5 x 500 µL of $F(ab')_2$ at 23 mg/ml. The sample was confirmed to be $F(ab')_2$ by reducing and non-reducing native and SDS-PAGE gels as well as by a positive antigen-catch ELISA assay targetting the k-chain followed by a negative assay targetting the Fc part of a human antibody molecule.

200 µl of Fab' at 23 mg/mL were diluted to 4 mL with 0.1 M Tris pH 8.0. 400 µL 100 mM DTT in 0.1 M Tris pH 8.0 were added to the 4 mL to provide a final concentration of 10 mM in DTT. The solution was incubated at room temperature for an hour, 30 µL of a 500 mM iodoacetamide solution in 0.1 M Tris pH 8.0 were added and the solution left for a further 30 minutes. The Fab' was dialyzed overnight against 20 mM Tris pH 8.0 and concentrated to 10 mg/mL for use in crystallization setups.

Crystals of uncomplexed Fab' grew from hanging drops of 5 mg/mL protein with 1.0 M ammonium sulfate at pH 5.8 as precipitant and grew as needles. Complexes were co-crystallized by adding a 3:1 ratio of peptide ELDKWAS to protein and incubating overnight before setting up as hanging drops of 5 mg/mL complex at pH 5.8, using 1.6 M ammonium sulfate at pH 7.0 as precipitant. The crystals grew in two days as large square bipyramids.

The sequence of the heavy and light variable domains has recently been published (ref. 10) and agrees with the one used in this study with a single correction at amino acid H110, which is a serine rather than a proline as originally stated. The full amino acid sequences of the variable and constant domains of the Fab' fragment are shown in Table 1 below (SEQ ID Nos: 6 and 7).

Crystals of the free Fab' belong to the space group $P2_12_12_1$ (unit cell: a=63.6 Å; b=76.4 Å; c=94.7 Å) and grow as needles. Crystals of the complex also adopt space group $P2_12_12_1$ (unit cell: a=59.0 Å; b=65.0 Å; c=175.6 Å) and grow as square bipyramids. Crystals were flash frozen for data collection. Data were collected on a Rigaku FR-C equipped with Molecular Structure Corp mirror optics and with a Mar345 image plate detector (Fab'2F5) and at the National Synchrotron Light Source in Brookhaven using a Mar30 detector (complex). Data were processed using DENZO and SCALEPACK (HKL Research).

Example 2

This Example describes the solution of the structure of the Fab'2F5 complexed and uncomplexed.

The structure of the Fab'2F5 complex was solved by molecular replacement (ref. 24) using PDB entry 1CLZ (ref. 25) minus sidechains and hypervariable loops as the search model. Constant and variable regions were used as independent models. The correct solution had a correlation coefficient of 35.3 (R=47.3%) using data to 3.3 Å. The CNS package (ref. 26) was used for refinement. A $2F_O$-F_C map generated after rigid body refinement of the polyalanine model allowed placement of most sidechains. After a cycle of simulated annealing, the hypervariable loops were included. Density for the peptide was clear at this point and could be fitted unambiguously. Following another cycle of annealing, positional and B-factor refinement, waters were

included where peaks of >3.5 were found in a difference map at an appropriate distance from a donor or acceptor atom.

The structure of the uncomplexed Fab'2F5 was solved by molecular replacement using the refined Fab'2F5 complex minus peptide as the search model. Correlation coefficient was 53.7, R=39.0%. Refinement followed the same procedure as for the complex. Statistics of data collection, processing and structure refinement are given in Table 2 below. The coordinates of the crystal structures have been deposited on April 9, 1999 in the Brookhaven Protein Data Bank under Accession Nos. 2F5A for the uncomplexed structure and 2F5B for the Fab'2F5-epitope complex.

Example 3

This Example demonstrates the utility of the three-dimensional structural information of Katinger's epitope (Examples 1 and 2) in the rational design of constraint peptide-based vaccines.

1. ECDKWCS CLP-634 (SEQ ID No: 3)

Based on the structural information, the Katinger's epitope may be locked with a disulfide bridge between positions 2 and 6 in the peptide ECDKWCS (CLP-634).

The linear peptide ECDKWCS was synthesised manually, on PAM support, by using a standard Solid Phase Peptide Synthesis methodology, with a t-Boc strategy. The crude peptide was cleaved off the resin by high-HF procedure. The crude material (54 mg) was dissolved in methanol (500 mL). 50 mM iodine in methanol was added dropwise, with stirring, until solution became pale-yellow. After 1 min of stirring, Dowex IX2-200 (acetate) resin (approx. 9 g) was added. The stirring was continued until solution became colourless. The resin was filtered off, 50 ml of water was added, the mixture was concentrated *in vacuo*, frozen and lyophilised. The crude cyclic peptide was purified by RP-HPLC.

2. EdapDKWES CLP-1309 (SEQ ID No: 4)

Based on the structural information, the Katinger's peptide also may be constrained with a lactam bond between positions 2 and 6 in the peptide EDapDKWES (CLP-1309).

The peptide: t-Boc-Glu(OBzl)-Dap(Fmoc)-Asp(OBzl)-Lys(2Cl-Cbz)-Trp(For)-Glu(OFm)-Ser(Bzl)-RESIN was assembled on a PAM solid support. Sidechains of Dap(2) and Glu(6) were subsequently deprotected by treatment with 25% piperidine. The sidechain cyclization was performed on the resin by adding four equivalents of HBTU and 8 equivalents of DIEA and shaking the mixture overnight. The cyclic peptide was cleaved off the resin by a standard HF procedure and the crude product was purified by RP-HPLC.

Abbreviations used in this Example are:

Dap = diaminopropionic acid

HBTU = O-Benzotriazolyl-N,N,N',N'-tetramethyluronium Hexafluorophosphate

DIEA = Di-isopropylethylamine

PAM = 4-Hydroxymethyl-phenylacetamidomethyl resin

Bzl = Benzyl

2-Cl-Cbz = 2-Chlorobenzyloxycarbonyl

For = Formyl

t-Boc = t-Butloxycarbonyl

Fmoc = Fluorenylmethoxycarbonyl

Fm = Fluorenylmethyl

Both peptides CLP-634 and CLP-1309 were found to be capable of forming an immuno-complex with monoclonal antibody 2F5 and were subsequently co-crystallized with the Fab' fragment. These results indicated that the constrained peptides may mimic the Katinger's epitope and would be useful as peptide-based vaccines.

Example 4

This Example demonstrates the formation of constrained peptide-carrier conjugates, for use as anti-HIV vaccines.

In order to conjugate the constrained peptide CLP-1309 (Example 3) to a carrier protein, a tetra-peptide Cys-Gly-Gly-Gly was linked to CLP-1309 at the N-terminal end and the resultant peptide was named as CLP-1491. Similarly, a tetra-peptide Gly-Gly-Gly-Cys was linked to CLP-1309 at the C-terminal end, and so the resultant peptide was named as CLP-1492.

Fifty microlitre of m-maleimidobenzoyl-N-hydroxysuccinimide (MBS, Pierce, 2 mg; 6.3 mmol in 1 mL of tetrahydrofuran or methanol) was added to a protein solution (approximately 10 mg of Hin47 or tetanus toxoid in 2 mL of 0.1 M phosphate buffer, pH 7.5). The reaction mixture was stirred for 30 min at room temperature under argon. The reaction mixture was applied to a Sephadex G-25 column (20 x 300 mm) equilibrated with 20 mM ammonium bicarbonate buffer, pH 7.2 and eluted with the same buffer. Elution was monitored by absorbance at 230 nm, and the eluted protein peak was pooled. The number of maleimide groups incorporated into the carrier was determined by adding excess 2-mercaptoethanol to the activated carrier-MBS and back-titrating the excess using a modified Ellman's method (ref. 31).

A general protocol for peptide-carrier conjugates has been described (ref. 32). Briefly, synthetic peptide (1 mg/mL) in degassed PBS buffer, pH 7.5 mixed with carrier-MBS (1 mg/mL). The reaction mixture was stirred overnight at room temperature under argon atmosphere. Excess N-ethyl-maleimide (Aldrich) was added to quench the reaction, and stirring continued for an additional hour. The insoluble precipitate was filtered off, and the filtrate was subjected to gel filtration chromatography using a Sephadex G-25 column. The peptide-carrier conjugate was collected. The molar ratio of carrier to peptide was determined by using amino acid analysis.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the crystal structure of the Fab'2F5 fragment has been elucidated, both in uncomplexed form and complexed with the epitope ELDKWAS, and peptides synthesized to correspond to the constrained structure of the peptide-protein interactions. Modifications are possible within the scope of this invention.

Table 1

ALQLTQSPSS LSASVGDRIT ITCRASQGVT SALAWYRQKP GSPPQLLIYD
ASSLESGVPS RFSGSGSGTE FTLTISTLRP EDFATYYCQQ LHFYPHTFGG
GTKVDVRRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC (SEQ ID No.: 6)

RITLKESGPP LVKPTQTLTL TCSFSGFSLS DFGVGVGWIR QPPGKALEWL
ALIYSDDDKR YSPSLNTRLT ITKDTSKNQV VLVMTRVSPV DTATYFCAHR
RGPTTLFGVP IARGPVNAMD VWGQGITVTI SSASTKGPSV FPLAPSSKST
SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV
VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT CPPCPAPELL
GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KAPPAPIEKT
ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG
QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
YTQKSLSLSP GK (SEQ ID No.: 7)

Table 2

Data Collection, Processing and Structure Refinement Parameters

Compound	Fab'2F5	Fab'2F5-ELDKWAS
Crystal system; space group	orthorhombic; $P2_12_12_1$	orthorhombic; $P2_12_12_1$
Unit cell (Å)	a=63.3 b=76.3 c=94.4	a=58.0; b=65.0, c=175.6
Resolution range (Å)	20.0 - 2.05	12.0 - 2.0
# of reflections	89376	118126
# unique reflections	28045	41062
Completeness; completeness top bin (%)	92; 93	90;92
R_{sym}; R_{sym} top bin (%)	7.0; 31.3	3.5; 16.6
-cutoff	0.0	1.0
% data in test set	5	5
# water molecules in model	268	357
R, R_{free}	0.23, 0.27	0.22, 0.25
Rmsd bonds (Å); angles (°)	0.007; 1.4	0.010; 1.5

Table 3

ATOM	2399	N	ALA	H	98	-.049	39.377	79.646	1.00	21.77
H										
ATOM	2400	CA	ALA	H	98	1.135	39.444	80.483	1.00	21.70
H										
ATOM	2401	CB	ALA	H	98	2.361	39.794	79.633	1.00	21.47
H										
ATOM	2402	C	ALA	H	98	.979	40.460	81.598	1.00	21.53
H										
ATOM	2403	O	ALA	H	98	.223	41.419	81.490	1.00	21.06
H										
ATOM	2404	N	HIS	H	99	1.731	40.229	82.660	1.00	21.37
H										
ATOM	2405	CA	HIS	H	99	1.719	41.072	83.841	1.00	21.17
H										
ATOM	2406	CB	HIS	H	99	1.956	40.169	85.059	1.00	21.35
H										
ATOM	2407	CG	HIS	H	99	2.229	40.897	86.336	1.00	21.04
H										
ATOM	2408	CD2	HIS	H	99	1.395	41.316	87.319	1.00	20.90
H										
ATOM	2409	ND1	HIS	H	99	3.504	41.224	86.746	1.00	21.12
H										
ATOM	2410	CE1	HIS	H	99	3.446	41.808	87.931	1.00	20.64
H										
ATOM	2411	NE2	HIS	H	99	2.179	41.876	88.301	1.00	20.95
H										
ATOM	2412	C	HIS	H	99	2.748	42.194	83.773	1.00	21.64
H										
ATOM	2413	O	HIS	H	99	3.831	42.026	83.207	1.00	21.32
H										
ATOM	2414	N	ARG	H	100	2.379	43.355	84.306	1.00	21.79
H										
ATOM	2415	CA	ARG	H	100	3.292	44.483	84.354	1.00	22.26
H										
ATOM	2416	CB	ARG	H	100	2.824	45.673	83.507	1.00	22.31
H										
ATOM	2417	CG	ARG	H	100	3.884	46.772	83.478	1.00	22.62
H										
ATOM	2418	CD	ARG	H	100	3.486	48.026	82.712	1.00	22.45
H										
ATOM	2419	NE	ARG	H	100	4.626	48.941	82.623	1.00	22.59
H										
ATOM	2420	CZ	ARG	H	100	4.569	50.179	82.133	1.00	22.62
H										
ATOM	2421	NH1	ARG	H	100	3.425	50.676	81.684	1.00	22.75
H										
ATOM	2422	NH2	ARG	H	100	5.674	50.910	82.055	1.00	23.15
H										
ATOM	2423	C	ARG	H	100	3.363	44.906	85.805	1.00	22.74
H										
ATOM	2424	O	ARG	H	100	2.337	45.128	86.460	1.00	22.03
H										
ATOM	2425	N	ARG	H	101	4.579	45.001	86.304	1.00	23.46
H										
ATOM	2426	CA	ARG	H	101	4.809	45.388	87.678	1.00	24.42
H										

ATOM	2427	CB	ARG	H	101	6.287	45.169	88.017	1.00	25.61
H										
ATOM	2428	CG	ARG	H	101	6.557	44.099	89.047	1.00	27.15
H										
ATOM	2429	CD	ARG	H	101	7.573	43.067	88.572	1.00	28.68
H										
ATOM	2430	NE	ARG	H	101	8.851	43.615	88.118	1.00	29.23
H										
ATOM	2431	CZ	ARG	H	101	9.867	42.858	87.697	1.00	29.78
H										
ATOM	2432	NH1	ARG	H	101	9.747	41.535	87.681	1.00	30.18
H										
ATOM	2433	NH2	ARG	H	101	11.001	43.410	87.276	1.00	29.91
H										
ATOM	2434	C	ARG	H	101	4.448	46.846	87.902	1.00	24.54
H										
ATOM	2435	O	ARG	H	101	4.544	47.668	86.996	1.00	23.94
H										
ATOM	2436	N	GLY	H	102	4.014	47.156	89.118	1.00	25.02
H										
ATOM	2437	CA	GLY	H	102	3.709	48.529	89.453	1.00	26.02
H										
ATOM	2438	C	GLY	H	102	4.957	49.055	90.136	1.00	27.10
H										
ATOM	2439	O	GLY	H	102	5.889	48.280	90.375	1.00	26.58
H										
ATOM	2440	N	PRO	H	103	5.031	50.357	90.449	1.00	27.97
H										
ATOM	2441	CD	PRO	H	103	4.057	51.435	90.215	1.00	28.46
H										
ATOM	2442	CA	PRO	H	103	6.218	50.901	91.111	1.00	29.02
H										
ATOM	2443	CB	PRO	H	103	5.863	52.379	91.269	1.00	28.75
H										
ATOM	2444	CG	PRO	H	103	4.982	52.630	90.056	1.00	28.56
H										
ATOM	2445	C	PRO	H	103	6.458	50.226	92.457	1.00	30.21
H										
ATOM	2446	O	PRO	H	103	5.515	49.927	93.185	1.00	30.26
H										
ATOM	2447	N	THR	H	104	7.723	49.967	92.772	1.00	31.28
H										
ATOM	2448	CA	THR	H	104	8.073	49.360	94.048	1.00	32.89
H										
ATOM	2449	CB	THR	H	104	9.586	49.042	94.115	1.00	32.77
H										
ATOM	2450	OG1	THR	H	104	9.898	48.014	93.167	1.00	33.00
H										
ATOM	2451	CG2	THR	H	104	9.987	48.579	95.514	1.00	32.60
H										
ATOM	2452	C	THR	H	104	7.720	50.366	95.141	1.00	33.71
H										
ATOM	2453	O	THR	H	104	7.978	51.559	94.994	1.00	33.67
H										
ATOM	2454	N	THR	H	105	7.123	49.889	96.225	1.00	35.02
H										
ATOM	2455	CA	THR	H	105	6.745	50.769	97.321	1.00	36.43
H										

ATOM 2456 CB THR H 105 5.217 50.723 97.589 1.00 36.53
H
ATOM 2457 OG1 THR H 105 4.837 49.399 97.990 1.00 36.95
H
ATOM 2458 CG2 THR H 105 4.437 51.116 96.334 1.00 36.64
H
ATOM 2459 C THR H 105 7.470 50.384 98.609 1.00 37.35
H
ATOM 2460 O THR H 105 7.892 49.242 98.773 1.00 37.48
H
ATOM 2461 N LEU H 106 7.625 51.354 99.506 1.00 38.42
H
ATOM 2462 CA LEU H 106 8.264 51.132 100.804 1.00 39.62
H
ATOM 2463 CB LEU H 106 9.633 51.813 100.877 1.00 39.53
H
ATOM 2464 CG LEU H 106 10.385 51.596 102.199 1.00 39.63
H
ATOM 2465 CD1 LEU H 106 10.643 50.107 102.396 1.00 39.65
H
ATOM 2466 CD2 LEU H 106 11.694 52.362 102.193 1.00 39.35
H
ATOM 2467 C LEU H 106 7.319 51.756 101.825 1.00 40.38
H
ATOM 2468 O LEU H 106 7.113 52.973 101.828 1.00 40.43
H
ATOM 2469 N PHE H 107 6.753 50.916 102.687 1.00 41.38
H
ATOM 2470 CA PHE H 107 5.784 51.366 103.679 1.00 42.27
H
ATOM 2471 CB PHE H 107 6.443 52.208 104.774 1.00 43.05
H
ATOM 2472 CG PHE H 107 7.522 51.488 105.525 1.00 43.75
H
ATOM 2473 CD1 PHE H 107 8.855 51.624 105.155 1.00 44.10
H
ATOM 2474 CD2 PHE H 107 7.202 50.645 106.585 1.00 44.17
H
ATOM 2475 CE1 PHE H 107 9.857 50.935 105.829 1.00 44.32
H
ATOM 2476 CE2 PHE H 107 8.195 49.948 107.265 1.00 44.42
H
ATOM 2477 CZ PHE H 107 9.527 50.094 106.887 1.00 44.38
H
ATOM 2478 C PHE H 107 4.736 52.194 102.946 1.00 42.37
H
ATOM 2479 O PHE H 107 4.355 53.276 103.390 1.00 42.68
H
ATOM 2480 N GLY H 108 4.298 51.681 101.799 1.00 42.27
H
ATOM 2481 CA GLY H 108 3.290 52.368 101.015 1.00 42.09
H
ATOM 2482 C GLY H 108 3.777 53.434 100.051 1.00 41.71
H
ATOM 2483 O GLY H 108 3.065 53.782 99.112 1.00 42.19
H
ATOM 2484 N VAL H 109 4.979 53.957 100.260 1.00 40.92
H

ATOM 2485 CA VAL H 109 5.491 54.996 99.373 1.00 40.10
H
ATOM 2486 CB VAL H 109 6.406 55.988 100.138 1.00 40.30
H
ATOM 2487 CG1 VAL H 109 6.868 57.097 99.209 1.00 40.21
H
ATOM 2488 CG2 VAL H 109 5.667 56.568 101.330 1.00 40.54
H
ATOM 2489 C VAL H 109 6.275 54.441 98.184 1.00 39.35
H
ATOM 2490 O VAL H 109 7.226 53.678 98.353 1.00 39.16
H
ATOM 2491 N PRO H 110 5.867 54.805 96.956 1.00 38.61
H
ATOM 2492 CD PRO H 110 4.728 55.654 96.569 1.00 38.51
H
ATOM 2493 CA PRO H 110 6.567 54.329 95.757 1.00 37.67
H
ATOM 2494 CB PRO H 110 5.728 54.922 94.629 1.00 37.96
H
ATOM 2495 CG PRO H 110 5.221 56.214 95.258 1.00 38.42
H
ATOM 2496 C PRO H 110 7.988 54.887 95.782 1.00 36.69
H
ATOM 2497 O PRO H 110 8.179 56.099 95.921 1.00 36.53
H
ATOM 2498 N ILE H 111 8.977 54.006 95.654 1.00 35.32
H
ATOM 2499 CA ILE H 111 10.377 54.419 95.692 1.00 34.04
H
ATOM 2500 CB ILE H 111 11.087 53.834 96.927 1.00 34.06
H
ATOM 2501 CG2 ILE H 111 10.441 54.361 98.204 1.00 34.21
H
ATOM 2502 CG1 ILE H 111 11.017 52.305 96.876 1.00 34.03
H
ATOM 2503 CD1 ILE H 111 11.776 51.607 97.990 1.00 33.88
H
ATOM 2504 C ILE H 111 11.180 54.009 94.463 1.00 33.02
H
ATOM 2505 O ILE H 111 12.367 54.322 94.365 1.00 32.88
H
ATOM 2506 N ALA H 112 10.551 53.296 93.536 1.00 31.78
H
ATOM 2507 CA ALA H 112 11.255 52.862 92.338 1.00 30.94
H
ATOM 2508 CB ALA H 112 12.149 51.670 92.667 1.00 30.98
H
ATOM 2509 C ALA H 112 10.300 52.496 91.213 1.00 30.17
H
ATOM 2510 O ALA H 112 9.394 51.681 91.398 1.00 30.19
H
ATOM 2511 N ARG H 113 10.506 53.091 90.046 1.00 29.21
H
ATOM 2512 CA ARG H 113 9.651 52.797 88.905 1.00 28.40
H
ATOM 2513 CB ARG H 113 9.199 54.100 88.239 1.00 28.78
H

ATOM 2514 CG ARG H 113 10.337 55.009 87.853 1.00 28.97
H
ATOM 2515 CD ARG H 113 9.850 56.258 87.132 1.00 29.05
H
ATOM 2516 NE ARG H 113 10.971 57.131 86.821 1.00 29.19
H
ATOM 2517 CZ ARG H 113 10.940 58.104 85.916 1.00 29.34
H
ATOM 2518 NH1 ARG H 113 9.831 58.339 85.217 1.00 28.91
H
ATOM 2519 NH2 ARG H 113 12.029 58.835 85.702 1.00 29.08
H
ATOM 2520 C ARG H 113 10.353 51.901 87.892 1.00 27.85
H
ATOM 2521 O ARG H 113 9.746 51.462 86.920 1.00 27.45
H
ATOM 2522 N GLY H 114 11.632 51.620 88.122 1.00 27.08
H
ATOM 2523 CA GLY H 114 12.367 50.768 87.203 1.00 26.56
H
ATOM 2524 C GLY H 114 11.655 49.456 86.897 1.00 26.06
H
ATOM 2525 O GLY H 114 11.588 49.036 85.738 1.00 25.97
H
ATOM 2526 N PRO H 115 11.132 48.763 87.918 1.00 25.66
H
ATOM 2527 CD PRO H 115 11.212 49.041 89.362 1.00 25.99
H
ATOM 2528 CA PRO H 115 10.432 47.497 87.700 1.00 25.02
H
ATOM 2529 CB PRO H 115 10.028 47.087 89.119 1.00 25.85
H
ATOM 2530 CG PRO H 115 9.921 48.435 89.838 1.00 26.45
H
ATOM 2531 C PRO H 115 9.239 47.534 86.734 1.00 24.10
H
ATOM 2532 O PRO H 115 8.808 46.495 86.252 1.00 23.75
H
ATOM 2533 N VAL H 116 8.700 48.710 86.446 1.00 22.92
H
ATOM 2534 CA VAL H 116 7.565 48.764 85.531 1.00 22.26
H
ATOM 2535 CB VAL H 116 6.730 50.062 85.719 1.00 21.84
H
ATOM 2536 CG1 VAL H 116 6.401 50.266 87.199 1.00 21.48
H
ATOM 2537 CG2 VAL H 116 7.472 51.255 85.150 1.00 20.99
H
ATOM 2538 C VAL H 116 8.022 48.696 84.066 1.00 22.08
H
ATOM 2539 O VAL H 116 7.198 48.513 83.166 1.00 22.38
H
ATOM 2540 N ASN H 117 9.327 48.824 83.826 1.00 21.63
H
ATOM 2541 CA ASN H 117 9.826 48.813 82.455 1.00 21.64
H
ATOM 2542 CB ASN H 117 11.071 49.697 82.338 1.00 21.90
H

ATOM 2543 CG ASN H 117 10.748 51.173 82.526 1.00 22.54
H
ATOM 2544 OD1 ASN H 117 9.686 51.630 82.116 1.00 22.65
H
ATOM 2545 ND2 ASN H 117 11.673 51.922 83.115 1.00 22.26
H
ATOM 2546 C ASN H 117 10.070 47.451 81.814 1.00 21.39
H
ATOM 2547 O ASN H 117 11.186 47.122 81.396 1.00 21.27
H
ATOM 2548 N ALA H 118 8.984 46.691 81.716 1.00 21.30
H
ATOM 2549 CA ALA H 118 8.964 45.364 81.123 1.00 21.19
H
ATOM 2550 CB ALA H 118 10.093 44.511 81.695 1.00 21.58
H
ATOM 2551 C ALA H 118 7.632 44.713 81.466 1.00 21.25
H
ATOM 2552 O ALA H 118 6.898 45.197 82.333 1.00 21.59
H
ATOM 2553 N MET H 119 7.329 43.630 80.759 1.00 21.14
H
ATOM 2554 CA MET H 119 6.153 42.814 81.012 1.00 21.00
H
ATOM 2555 CB MET H 119 5.413 42.486 79.712 1.00 21.35
H
ATOM 2556 CG MET H 119 4.782 43.691 79.004 1.00 21.59
H
ATOM 2557 SD MET H 119 3.738 44.767 80.053 1.00 22.00
H
ATOM 2558 CE MET H 119 4.880 45.836 80.681 1.00 24.35
H
ATOM 2559 C MET H 119 6.907 41.594 81.542 1.00 21.33
H
ATOM 2560 O MET H 119 7.499 40.829 80.773 1.00 21.24
H
ATOM 2561 N ASP H 120 6.894 41.430 82.858 1.00 21.43
H
ATOM 2562 CA ASP H 120 7.679 40.381 83.500 1.00 21.62
H
ATOM 2563 CB ASP H 120 8.014 40.819 84.932 1.00 21.73
H
ATOM 2564 CG ASP H 120 6.806 40.826 85.840 1.00 22.35
H
ATOM 2565 OD1 ASP H 120 5.661 40.878 85.330 1.00 21.92
H
ATOM 2566 OD2 ASP H 120 7.011 40.807 87.075 1.00 21.94
H
ATOM 2567 C ASP H 120 7.209 38.931 83.499 1.00 21.67
H
ATOM 2568 O ASP H 120 8.020 38.027 83.688 1.00 21.12
H

Table 4

ELDKWAS:

```
ATOM  3373  CB  GLU P   1     .169  60.111  75.304  1.00 29.50   P
ATOM  3374  CG  GLU P   1    -.450  58.935  76.069  1.00 30.79   P
ATOM  3375  CD  GLU P   1   -1.151  57.917  75.185  1.00 31.68   P
ATOM  3376  OE1 GLU P   1    -.571  57.477  74.172  1.00 32.86   P
ATOM  3377  OE2 GLU P   1    2.288  57.530  75.519  1.00 31.76   P
ATOM  3378  C   GLU P   1    2.442  59.065  75.475  1.00 27.76   P
ATOM  3379  O   GLU P   1    2.777  57.902  75.230  1.00 27.40   P
ATOM  3380  N   GLU P   1    1.201  58.964  73.347  1.00 28.40   P
ATOM  3381  CA  GLU P   1    1.473  59.802  74.549  1.00 28.51   P
ATOM  3382  N   LEU P   2    2.882  59.739  76.537  1.00 27.14   P
ATOM  3383  CA  LEU P   2    3.825  59.156  77.497  1.00 26.40   P
ATOM  3384  CB  LEU P   2    4.343  60.235  78.462  1.00 26.88   P
ATOM  3385  CG  LEU P   2    5.264  61.329  77.913  1.00 27.33   P
ATOM  3386  CD1 LEU P   2    5.473  62.406  78.981  1.00 27.63   P
ATOM  3387  CD2 LEU P   2    6.590  60.720  77.491  1.00 27.68   P
ATOM  3388  C   LEU P   2    3.239  58.008  78.317  1.00 25.81   P
ATOM  3389  O   LEU P   2    2.049  58.000  78.625  1.00 25.51   P
ATOM  3390  N   ASP P   3    4.089  57.047  78.676  1.00 24.98   P
ATOM  3391  CA  ASP P   3    3.676  55.898  79.480  1.00 24.32   P
ATOM  3392  CB  ASP P   3    4.873  54.973  79.733  1.00 23.70   P
ATOM  3393  CG  ASP P   3    4.531  53.803  80.642  1.00 23.27   P
ATOM  3394  OD1 ASP P   3    3.595  53.040  80.302  1.00 22.76   P
ATOM  3395  OD2 ASP P   3    5.191  53.643  81.693  1.00 21.86   P
ATOM  3396  C   ASP P   3    3.109  56.356  80.824  1.00 24.44   P
ATOM  3397  O   ASP P   3    3.351  57.484  81.263  1.00 24.24   P
ATOM  3398  N   LYS P   4    2.380  55.466  81.489  1.00 24.58   P
ATOM  3399  CA  LYS P   4    1.784  55.778  82.784  1.00 25.00   P
ATOM  3400  CB  LYS P   4    1.079  54.543  83.350  1.00 24.68   P
ATOM  3401  CG  LYS P   4     .247  54.779  84.613  1.00 24.80   P
ATOM  3402  CD  LYS P   4    -.464  53.485  85.037  1.00 24.50   P
ATOM  3403  CE  LYS P   4   -1.508  53.723  86.133  1.00 24.83   P
ATOM  3404  NZ  LYS P   4   -2.572  54.671  85.678  1.00 24.26   P
ATOM  3405  C   LYS P   4    2.816  56.253  83.806  1.00 25.53   P
ATOM  3406  O   LYS P   4    2.528  57.124  84.622  1.00 25.08   P
ATOM  3407  N   TRP P   5    4.020  55.693  83.753  1:00 25.97   P
ATOM  3408  CA  TRP P   5    5.030  56.046  84.743  1.00 27.09   P
ATOM  3409  CB  TRP P   5    5.639  54.756  85.307  1.00 26.62   P
```



```
ATOM  3410  CG  TRP P   5    4.580  53.754  85.684  1.00 26.36   P
ATOM  3411  CD2 TRP P   5    3.646  53.863  86.766  1.00 26.15   P
ATOM  3412  CE2 TRP P   5    2.774  52.752  86.682  1.00 25.96   P
ATOM  3413  CE3 TRP P   5    3.461  54.795  87.798  1.00 26.24   P
ATOM  3414  CD1 TRP P   5    4.247  52.607  85.006  1.00 26.28   P
ATOM  3415  NE1 TRP P   5    3.164  52.003  85.602  1.00 25.88   P
ATOM  3416  CZ2 TRP P   5    1.728  52.545  87.595  1.00 25.85   P
ATOM  3417  CZ3 TRP P   5    2.415  54.593  88.706  1.00 26.20   P
ATOM  3418  CH2 TRP P   5    1.564  53.477  88.597  1.00 25.91   P
ATOM  3419  C   TRP P   5    6.137  56.995  84.280  1.00 27.96   P
ATOM  3420  O   TRP P   5    7.123  57.182  84.985  1.00 27.77   P
ATOM  3421  N   ALA P   6    5.967  57.598  83.107  1.00 29.24   P
ATOM  3422  CA  ALA P   6    6.957  58.534  82.571  1.00 30.79   P
ATOM  3423  CB  ALA P   6    6.738  58.733  81.077  1.00 30.55   P
ATOM  3424  C   ALA P   6    6.919  59.890  83.277  1.00 32.11   P
ATOM  3425  O   ALA P   6    5.904  60.273  83.848  1.00 32.54   P
ATOM  3426  N   SER P   7    8.040  60.601  83.213  1.00 33.55   P
ATOM  3427  CA  SER P   7    8.206  61.923  83.812  1.00 35.02   P
ATOM  3428  CB  SER P   7    7.007  62.821  83.481  1.00 35.56   P
ATOM  3429  OG  SER P   7    6.922  63.058  82.085  1.00 36.31   P
ATOM  3430  C   SER P   7    8.388  61.868  85.317  1.00 35.70   P
ATOM  3431  O   SER P   7    9.555  61.945  85.772  1.00 35.92   P
ATOM  3432  OT  SER P   7    7.357  61.724  86.013  1.00 36.58   P
```

Table 5

ELDRWAS:

ATOM	3265	CB	GLU	P	1	.001	59.852	75.796	1.00	71.00	P
ATOM	3266	CG	GLU	P	1	-.479	58.562	76.462	1.00	71.58	P
ATOM	3267	CD	GLU	P	1	-1.144	57.609	75.494	1.00	71.95	P
ATOM	3268	OE1	GLU	P	1	-.554	57.311	74.431	1.00	72.48	P
ATOM	3269	OE2	GLU	P	1	-2.260	57.134	75.803	1.00	71.87	P
ATOM	3270	C	GLU	P	1	2.326	58.990	75.760	1.00	36.82	P
ATOM	3271	O	GLU	P	1	2.717	57.867	75.436	1.00	36.76	P
ATOM	3272	N	GLU	P	1	.985	59.009	73.662	1.00	37.23	P
ATOM	3273	CA	GLU	P	1	1.270	59.720	74.941	1.00	37.14	P
ATOM	3274	N	LEU	P	2	2.775	59.627	76.833	1.00	33.88	P
ATOM	3275	CA	LEU	P	2	3.783	59.034	77.702	1.00	33.45	P
ATOM	3276	CB	LEU	P	2	4.389	60.114	78.611	1.00	61.37	P
ATOM	3277	CG	LEU	P	2	5.316	61.181	78.000	1.00	61.47	P
ATOM	3278	CD1	LEU	P	2	5.506	62.346	78.978	1.00	61.51	P
ATOM	3279	CD2	LEU	P	2	6.659	60.540	77.642	1.00	61.59	P
ATOM	3280	C	LEU	P	2	3.249	57.876	78.568	1.00	33.17	P
ATOM	3281	O	LEU	P	2	2.140	57.937	79.109	1.00	32.99	P
ATOM	3282	N	ASP	P	3	4.054	56.821	78.684	1.00	36.78	P
ATOM	3283	CA	ASP	P	3	3.700	55.666	79.496	1.00	36.51	P
ATOM	3284	CB	ASP	P	3	4.892	54.727	79.664	1.00	27.42	P
ATOM	3285	CG	ASP	P	3	4.583	53.569	80.597	1.00	27.10	P
ATOM	3286	OD1	ASP	P	3	3.676	52.778	80.258	1.00	26.93	P
ATOM	3287	OD2	ASP	P	3	5.235	53.460	81.668	1.00	26.53	P
ATOM	3288	C	ASP	P	3	3.285	56.155	80.868	1.00	36.57	P
ATOM	3289	O	ASP	P	3	3.595	57.280	81.245	1.00	36.49	P
ATOM	3290	N	ARG	P	4	2.628	55.288	81.629	1.00	47.13	P
ATOM	3291	CA	ARG	P	4	2.150	55.639	82.957	1.00	47.37	P
ATOM	3292	CB	ARG	P	4	1.309	54.495	83.516	1.00	57.30	P
ATOM	3293	CG	ARG	P	4	.545	54.865	84.764	1.00	57.28	P
ATOM	3294	CD	ARG	P	4	-.201	53.678	85.351	1.00	57.26	P
ATOM	3295	NE	ARG	P	4	-1.066	54.115	86.436	1.00	50.30	P
ATOM	3296	CZ	ARG	P	4	-1.736	53.309	87.256	1.00	50.30	P
ATOM	3297	NH1	ARG	P	4	-1.646	51.994	87.118	1.00	50.30	P
ATOM	3298	NH2	ARG	P	4	-2.495	53.822	88.227	1.00	50.30	P
ATOM	3299	C	ARG	P	4	3.238	56.014	83.971	1.00	47.65	P
ATOM	3300	O	ARG	P	4	3.016	56.861	84.840	1.00	47.39	P
ATOM	3301	N	TRP	P	5	4.412	55.402	83.873	1.00	41.46	P

ATOM	3302	CA	TRP	P	5	5.460	55.724	84.829	1.00	41.97	P
ATOM	3303	CB	TRP	P	5	6.039	54.431	85.387	1.00	45.39	P
ATOM	3304	CG	TRP	P	5	4.981	53.415	85.744	1.00	45.32	P
ATOM	3305	CD2	TRP	P	5	4.092	53.454	86.870	1.00	45.24	P
ATOM	3306	CE2	TRP	P	5	3.257	52.319	86.781	1.00	45.24	P
ATOM	3307	CE3	TRP	P	5	3.920	54.340	87.948	1.00	45.31	P
ATOM	3308	CD1	TRP	P	5	4.655	52.292	85.041	1.00	45.27	P
ATOM	3309	NE1	TRP	P	5	3.623	51.627	85.657	1.00	45.13	P
ATOM	3310	CZ2	TRP	P	5	2.266	52.044	87.724	1.00	45.22	P
ATOM	3311	CZ3	TRP	P	5	2.931	54.064	88.891	1.00	45.30	P
ATOM	3312	CH2	TRP	P	5	2.117	52.924	88.769	1.00	45.34	P
ATOM	3313	C	TRP	P	5	6.582	56.618	84.264	1.00	42.36	P
ATOM	3314	O	TRP	P	5	7.669	56.695	84.834	1.00	42.32	P
ATOM	3315	N	ALA	P	6	6.296	57.305	83.157	1.00	47.84	P
ATOM	3316	CA	ALA	P	6	7.267	58.192	82.512	1.00	48.51	P
ATOM	3317	CB	ALA	P	6	6.977	58.286	81.026	1.00	39.87	P
ATOM	3318	C	ALA	P	6	7.290	59.597	83.117	1.00	49.00	P
ATOM	3319	O	ALA	P	6	6.372	60.000	83.838	1.00	49.16	P
ATOM	3320	N	SER	P	7	8.349	60.336	82.795	1.00	52.63	P
ATOM	3321	CA	SER	P	7	8.551	61.700	83.282	1.00	53.25	P
ATOM	3322	CB	SER	P	7	7.283	62.531	83.064	1.00	91.37	P
ATOM	3323	OG	SER	P	7	7.464	63.854	83.541	1.00	91.74	P
ATOM	3324	C	SER	P	7	8.937	61.727	84.765	1.00	53.52	P
ATOM	3325	O	SER	P	7	10.153	61.808	85.062	1.00	53.79	P
ATOM	3326	OT	SER	P	7	8.026	61.637	85.617	1.00	92.11	P

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CLAIMS

What we claim is:

1. An isolated crystal of the Fab' fragment of monoclonal antibody 2F5.

2. The isolated crystal of claim 1 consisting of molecules having the three-dimensioned structure represented by Figure 1.

3. The isolated crystal of claim 1 consisting of molecules having the parameters defined in Table 2.

4. The isolated crystal of claim 3 consisting of molecules having a space group $P2_12_12_1$, with said cell dimensions $\underline{a}$ = 63.6 Å, $\underline{b}$ = 76.4 Å and $\underline{c}$ = 93.4 Å.

5. The isolated crystal of claim 1 consisting of molecules having a third hypervariable (V3) loop of the heavy chain comprising amino acid residues H98 to H120, as seen in Table 1, having a three-dimensional structure as shown in Figure 4.

6. The isolated crystal of claim 5 consisting of molecules wherein said V3 loop has the atomic coordinates shown in Table 3.

7. The isolated crystal of claim 1 consisting of molecules having the atomic coordinates deposited with the Protein Data Bank under Accession number 2F5A.

8. An isolated crystal of the Fab' fragment of monoclonal antibody 2F5 complexed with a peptide having the amino acid sequence ELDKWAS (SEQ ID No.: 1) or a functional analog thereof.

9. The isolated crystal of claim 8 consisting of molecules having a structure at the binding site of the peptide to the Fab' fragment as shown in Figure 3.

10. The isolated crystal of claim 8 consisting of molecules having the parameters defined in Table 2.

11. The isolated crystal of claim 10 consisting of molecules having a space group $P2_12_12_1$, with unit cell dimensions $\underline{a}$ = 58.0 Å, $\underline{b}$ = 65.0 Å and $\underline{c}$ = 175.6 Å.

12. The isolated crystal of claim 8 wherein said functional analog of said amino acid sequence ELDKWAS is selected from the group consisting of one in which lysine is replaced by arginine and one in which tryptophan is replaced by an amino acid selected from the group consisting of tyrosine, phenylalanine and uncharged histadine.

13. The isolated crystal of claim 8 wherein said peptide is ELDKWAS.

14. The isolated crystal of claim 13 wherein the Fab'2F5 : ELDKWAS complex has the atomic coordinates of Table 4.

15. The isolated crystal of claim 8 wherein said peptide is ELDRWAS (SEQ ID No: 2).

16. The isolated crystal of claim 15 wherein said Fab'2F5 : ELDRWAS complex has the atomic coordinates of Table 5.

17. The isolated crystal of claim 8 consisting of molecules having the atomic coordinates deposited with the Protein Data Bank under Accession number 2F5B.

18. A synthetic peptide which binds to monoclonal antibody 2F5 and which is constrained to provide a three-dimensional structure corresponding to that for the peptide ELDKWAS (SEQ ID No.: 1) shown in Figure 3.

19. The synthetic peptide of claim 18 which contains the amino acid sequence DKW or a functional analog thereof constrained in the slightly distorted ß-turn configuration of said three-dimensional structure with the tryptophan and lysine sidechains stacked and parallel.

20. The synthetic peptide of claim 19 wherein at least one of said tryptophan and lysine amino acids is substituted by an amino acid which retains the peptide-protein interactions shown in Figure 3.

21. The synthetic peptide of claim 20 wherein said lysine residues is replaced by arginine.

22. The synthetic peptide of claim 20 wherein said tryptophan is replaced by tyrosine, phenylalanine or uncharged histadine.

23. The synthetic peptide of claim 20 wherein said lysine residue is replaced by arginine and said tryptophan is replaced by tyrosine, phenylalanine or uncharged histadine.

24. The synthetic peptide of claim 19 wherein said peptide contains a disulphide bridge to maintain said amino acid sequence DKW or functional analog thereof in the respective orientation of the amino acid residues as shown in Figure 3.

25. The synthetic peptide of claim 24 wherein said peptide has the amino acid sequence ECDKWCS (SEQ ID No.: 3) and said disulphide bridge is established between said cysteine (C) residues.

26. The synthetic peptide of claim 19 wherein said peptide contains a lactain bond to maintain said amino acid sequence DKW or functional analog thereof in the respective orientation of the amino acid residues as shown in Figure 3.

27. The synthetic peptide of claim 26 wherein said peptide has the formula EDapDKWES (SEQ ID No.: 5) and said lactam bond is formed between the Dap and glutamate (E) residues.

28. The synthetic peptide of claim 18 which is linked to a carrier protein.

29. A method of making a peptide binding to monoclonal antibody 2F5, which comprises:

co-crystallizing a Fab' fragment of the monoclonal antibody 2F5 with a peptide having the amino acid sequence ELDKWAS (SEQ ID No: 1) or functional analog thereof to form a crystalline complex,

analyzing the crystalline complex to determine the three-dimensional orientation of the bound peptide in relation to the Fab' fragment, and

synthesizing a peptide containing at least amino acids DKW or functional analogs thereof constrained in the determined three-dimensional orientation.

30. The method of claim 29 wherein said functional analog of the peptide having the amino acid sequence ELDKWAS has the amino acid sequence ELDRWAS (SEQ ID No.: 2).

FIG.1

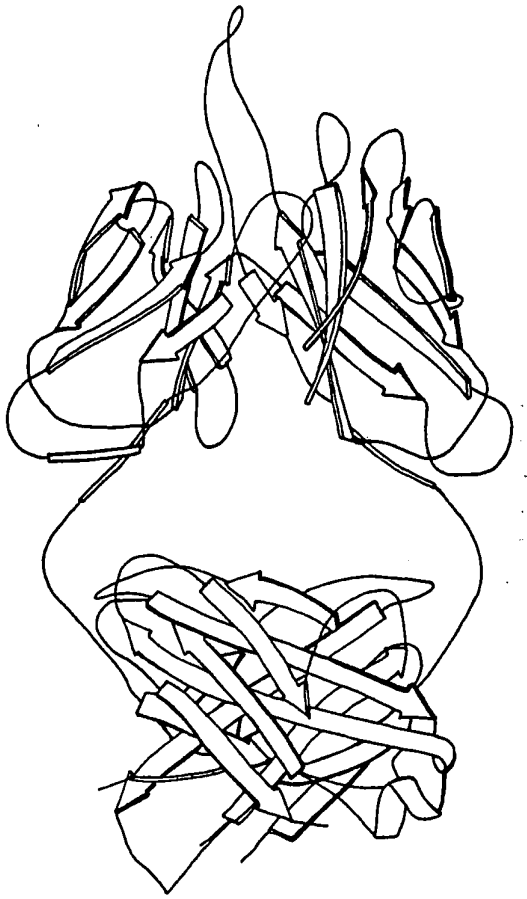


FIG.2

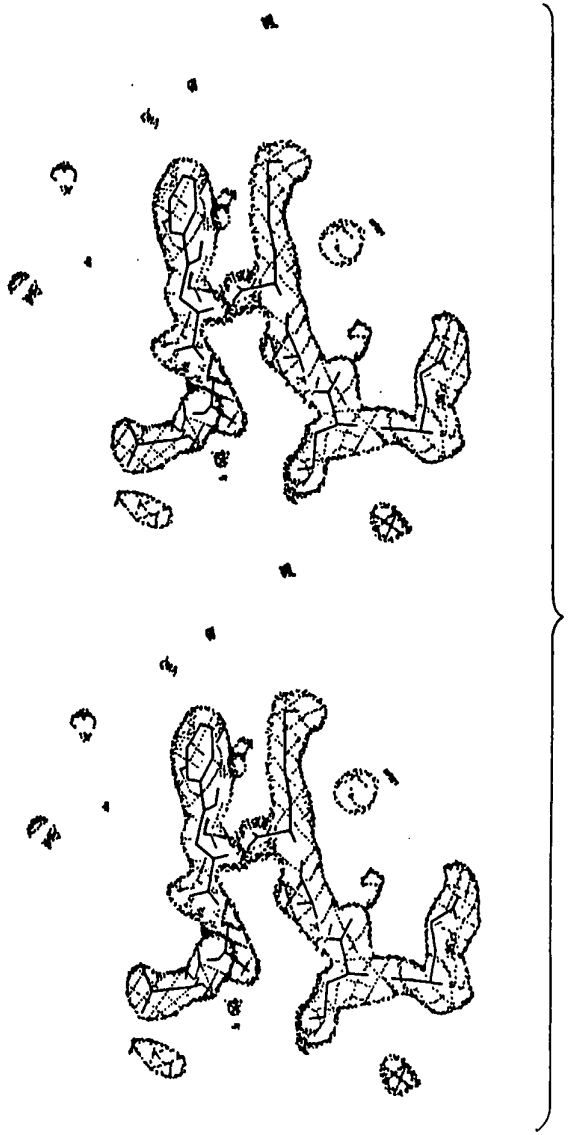


FIG.3

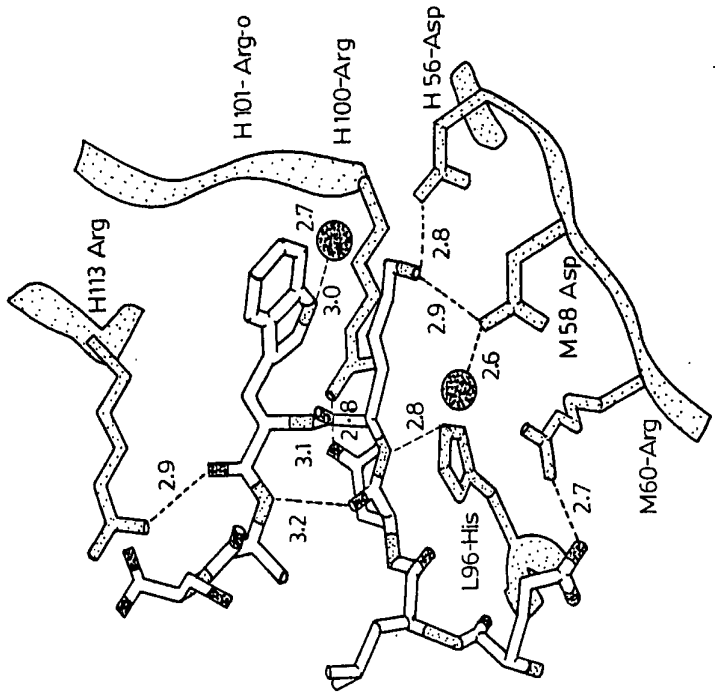
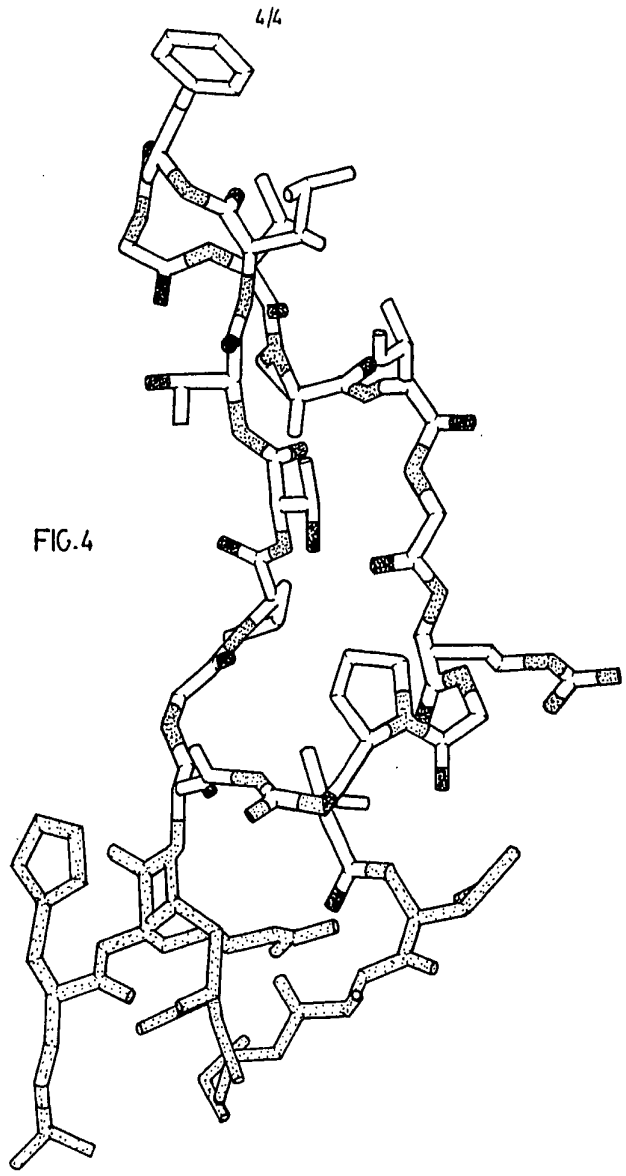

H 101- Arg-o
H 100-Arg
H 56-Asp
H113 Arg
M 58 Asp
M60-Arg
L96-His
2.7
3.0
2.8
2.9
2.6
2.8
2.8
3.1
2.9
3.2
2.7

FIG.4



INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/CA 00/00358

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/16 C07K16/10 C07K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 02273 A (SCRIPPS RESEARCH INST) 1 February 1996 (1996-02-01) abstract; figure 6 page 102, line 10 - line 17	1-7
Y	MUSTER T. ET AL: "Cross-Neutralizing Activity against Divergent Human Immunodeficiency Virus Type I Isolates Induced by the gp41 Sequence ELDKWAS." J. VIROL., vol. 68, no. 6, 1994, pages 4031-4034, XP000654602 abstract page 4031, column 1, paragraph 1 page 4033, column 2, paragraphs 2,3	1-17,25, 29,30

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2000	24/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montrone, M

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INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/CA 00/00358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PURTSCHER M. ET AL: "Restricted antigenic varaibility of the epitope recognized by the neutralizing gp41 antibody 2F5." AIDS, vol. 10, 1996, pages 587-593, XP000916358 abstract page 589, column 1, paragraph 3 page 590, column 2, paragraph 4 -page 591, column 2, paragraph 1; figure 1 page 592, column 1, paragraph 2 - paragraph 3	1-17,25, 29,30
Y	CONLEY A.J. ET AL: "Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody." PNAS, vol. 91, 1994, pages 3348-3352, XP000652271 abstract page 3351, column 1, paragraph 3 -column 2, paragraph 1; figure 3	1-17,25, 29,30
Y	JEFFREY P.D. ET AL: "The X-ray structure of an anti-tumour antibody in complex with antigen" NATURE STRUCTURAL BIOLOGY, vol. 2, 1995, pages 466-471, XP000915894 cited in the application abstract page 466, column 2, paragraph 2	7-17
Y	US 5 831 034 A (STEINDL FRANZ ET AL) 3 November 1998 (1998-11-03) abstract column 1, line 18 - line 24 column 1, line 65 claims 1-4	1-7
Y	WO 95 07354 A (MUSTER THOMAS ;KATINGER HERMANN (AT); POLYMUN SCIENT IMMUNBIO FORS) 16 March 1995 (1995-03-16) abstract page 3, line 25 - line 33 page 4, line 10 - line 20 page 11, line 7 - line 22	1-17,25, 29,30

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INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 00/00358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COOK J. ET AL: "Recombinant Antibodies with Conformational Constrained HIV Type I Epitope Inserts Elicit Glycoprotein 160-Specific Antibody Responses in Vivo" AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 13, 1997, pages 449-460, XP000916356 abstract page 450, column 1, paragraph 2 page 452, column 2, paragraph 1; figure 1 page 456, column 2, paragraph 2 page 457, column 1, paragraph 3	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No PCT/CA 00/00358

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		US 5753503 A	19-05-1998
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